24 NICOTINE-INDUCED CONTRACTION OF TRACHEAL SMOOTH MUSCLE *IN VITRO* REQUIRES PRESENCE OF TRACHEAL EPITHELIUM.

M. Lang, H.-L. Hahn

Medizinische Poliklinik, University of Würzburg, Klinikstr. 8, 8700 WÜRZBURG, W. Germany.

The tracheobronchial epithelium can inhibit airway smooth muscle contraction by various mechanisms but epithelial cells in culture have also been shown to release mediators capable of causing bronchoconstriction. In order to assess the mechanism of and the rôle of the epithelium in, nicotine-induced bronchoconstriction we suspended tracheal smooth muscle strips or tracheal rings from 22 ferrets (1247±526 g, x±SD) anaesthetized with 66±19 $mg \cdot kg^{-1}$ pentobarbital in organ baths filled with medium M 199 and aerated with 95% O_2 and 5% CO_2. Muscle tension was measured with force transducers (FT03, Grass). Signals were amplified and recorded with an eight channel recorder (Hellige). Baseline smooth muscle tension was set at a level leading to maximal contraction on subsequent addition of acetylcholine (ACh, 10^{-6}M). To determine magnitude and mechanism of nicotine-induced bronchoconstriction we constructed a cumulative dose response curve to ACh (10^{-8} - 10^{-4} M) after which each ring received a single dose of nicotine ($3 \cdot 10^{-5}$ - $3 \cdot 10^{-4}$ M) in the presence or absence of hexamethonium (10^{-5} M, 10^{-6} M), tetrodotoxin (10^{-6} M) or atropine (10^{-8}, 10^{-7}, or 10^{-6} M). To determine the rôle of the epithelium in nicotine-induced bronchoconstriction we removed the epithelium from alternate tracheal strips by stroking them gently with a scalpel blade moved backwards (i.e., away from the cutting edge). This procedure also removed some subepithelial connective tissue (verified histologically). Dose response curves to ACh and nicotine were established for pairs of tissues with and without epithelium, each pair receiving only one dose of nicotine.

Nicotine induced brief muscle contractions not exceeding 25% of the ACh-induced maximum. Contractions were blocked by hexamethonium, atropine (10^{-7} M, 10^{-6} M), and were abolished or inhibited strongly by tetrodotoxin, suggesting the involvement of nicotinic ganglionic and/or neuronal receptors and of muscarinic smooth muscle receptors. Removal of epithelium strongly inhibited contractions at concentrations of nicotine $> 3 \cdot 10^{-5}$ M which completely removed any dose response effect. ACh-induced contractions were not changed by removal of epithelium, demonstrating smooth muscle integrity.

We conclude that nicotine acts on ganglionic and/or neuronal nicotinic receptors to initiate muscle contraction by a neural pathway. The effect can be blocked either by the neuronal blocker tetrodotoxin or by atropine which antagonizes the contracting effect on smooth muscle of Ach released from the nerve ending. Removal of epithelium and/or of portions of the submucosal tissue inhibits or blocks nicotine-induced smooth muscle contraction presumably by removing or interrupting nerves running in or close to the epithelium. This reveals one more mechanism by which the removal of epithelium can influence smooth muscle contraction. The epithelium and the adjacent subepithelial tissue influences airway smooth muscle contraction not only by generating relaxing or contracting factors or by acting as a diffusion barrier but also by acting as a nerve conduit. These could be either efferent cholinergic nerves or afferent (e.g., peptide containing) nerves interacting locally with the efferent pathway.